of the heavy chain or light chain contained in an immunoglobulin. Also in this case, a chimeric protein in which N terminus side of the protein is an integrin molecule and then connected to an immunoglobulin side by side is preferable. In either case of α chain or β chain, a chimeric protein bound to the heavy chain of an immunoglobulin is preferable.

Please replace the paragraph bridging pages 13 and 14 with the following:

A DNA coding for the α chain and β chain of an integrin can be obtained using the information of known cDNA sequences by such a method as gene amplification based on the PCR method, cDNA cloning or DNA synthesis. For example, the DNA sequences of α4 and β1 are already reported in literature (Takada, Y. et al., EMBO J., 8, 1361-1368 (1989), Scott Argraves, W. et al., J. Cell Biol., 105, 1183-1190 (1987)). A DNA coding for the α chain and β chain of an integrin can also be obtained by ~~the~~ expression cloning using an antibody, etc. For binding to a DNA coding for the constant region of an immunoglobulin, it is desirable to take out a DNA coding for the extracellular portions only of the α chain and β chain of an integrin. For this purpose, it is preferable to use the PCR method and DNA synthesis. The extracellular portion of either an α chain or β chain refers to the polypeptide sequence on the N terminus side from the portion speculated to be the transmembrane portion. Its partial sequence can also be used as far as the ligand binding capability is retained, but it is preferable to use most of the portion considered to be an extracellular region. For taking out a DNA, it is necessary to adjust for adaptation of frames after

linking to a DNA coding for an immunoglobulin. For example, this can be achieved by modifying the primer when a DNA fragment is taken out by the PCR method. In this case, it is desirable to design for ensuring that amino acid modification is not caused by the base substitution of the primer. However, amino acid substitution is allowed ~~as far as~~if the function of the chimeric protein is not changed. For obtaining a DNA by chemical synthesis, the purpose can be achieved by designing a sequence to ensure the linking to a DNA coding for an immunoglobulin. In the case of cDNA, a DNA capable of being bound to a DNA coding for an immunoglobulin can be prepared by using DNA fragmentation and a synthetic DNA.

Please replace the paragraph bridging pages 16 and 17 with the following:

If the obtained recombinant vector is transfected into a cell, a cell capable of producing an integrin-immunoglobulin chimeric protein heterodimer complex can be obtained. In this case it is preferable to use an animal derived cell as a host. For example, a COS cell (simian renal cell), CHO cell (Chinese Hamster ovarian cell), Sf9 (insect cell), etc. are generally used as hosts. Furthermore, myeloma cells such as P3UI and Y3 can also be used. Other established cell lines and cloned cells can also be used, but the cells used as hosts are not limited to them. In the present invention, it is preferable to use a CHO cell.

Please replace the paragraph bridging pages 18 and 19 with the following:

In any transfection method and any combination of vectors, it is important to select a cell which is transfected by the two

recombinant vectors and produces a chimeric protein consisting of an α chain and the heavy chain or light chain of an immunoglobulin and a chimeric protein consisting of a β chain and the heavy chain or light chain of an immunoglobulin simultaneously almost ~~by~~in the same amounts. This can be achieved by measuring the amounts of the chimeric protein consisting of an α chain and the heavy chain or light chain of an immunoglobulin and a chimeric protein consisting of a β chain and the heavy chain or light chain of an immunoglobulin produced in the cultured supernatant solution of the cell transfected by the recombinant vectors. For measurement, for example, the transfected cell can be cultured in a medium containing ^{35}S according to any publicly known method, for labeling the proteins, and the amounts of the chimeric protein consisting of an α chain and the heavy chain or light chain of an immunoglobulin and a chimeric protein consisting of a β chain and the heavy chain or light chain of an immunoglobulin existing in the cultured supernatant solution can be estimated by immunoprecipitation using an anti-α chain antibody or an anti-β chain antibody respectively. As another method, the amounts of the chimeric protein consisting of an α chain and the heavy chain or light chain of an immunoglobulin and a chimeric protein consisting of a β chain and the heavy chain or light chain of an immunoglobulin existing in the cultured supernatant solution can be estimated according to the ELISA method using an anti-human immunoglobulin antibody and an anti-α chain antibody or an anti-β chain antibody. ~~Anyway, it~~It is

preferable to select a clone which produces almost the same large amounts of the chimeric proteins of the α and β chains in the cultured supernatant solution, for preparing an integrin-immunoglobulin chimeric protein heterodimer complex. The methods for labeling proteins, the methods of immunoprecipitation and the general methods of ELISA are described in a published book ("Antibody" Harlow, E., and Lane, D. (1988), Cold Spring Harbor Lab. Press, New York), but the methods are not limited to them. Any other method can also be used for detecting chimeric proteins.

Please replace the first full paragraph on page 21 with the following:

An integrin-immunoglobulin chimeric protein heterodimer complex can be purified by an established method using a protein A column chromatography by use of the nature of the immunoglobulin portion. Furthermore, affinity chromatography using an antibody against the α or β chain can also be used. Moreover, the purification can also be achieved by affinity chromatography with a ligand bound to a carrier. General chromatographic methods can also be used in combination for the purification. If publicly known cases are applied in which integrin molecules are purified by these methods (Pytela, R. et al., Methods Enzymol., 144, 475-489 (1987), Santoro, S. A. et al., Biochem. Biophys. Res. Comm., 153, 217-223 (1988), Charo, I.F. et al., J. Cell Biol., 111, 2795-2800 (1990), Makarem, R. et al., J. Biol. Chem., 269, 4005-4011 (1994), Pfaff, M. et al., Eur. J. Immunol., 225, 975-984 (1994), Gulino, D. et al., Eur. J. Biochem., 227, 108-115 (1995), etc.) ~~are~~

~~applied~~, the purification of an integrin-immunoglobulin chimeric protein heterodimer complex can be achieved.

Please replace the paragraph bridging pages 35 and 36 with the following:

The template DNA, primers, dNTPs (an equimolar mixture of dATP, dCTP, dGTP and dTTT) and Taq polymeraze (Takara) were mixed in a PCR buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl^2$, 0.01% gelatin, pH 8.3), and in a thermal cycler (Perkin Elmer Cetus), the mixture was treated at 94°C for 1 minute for DNA denaturation, at 58 °C for 2 minutes for annealing the primers and at 72°C for 3 minutes for elongating the primers. This cycle of treatment was performed for 30 cycles. The amplified DNA was digested by restriction enzymes BamH I and Xba I, and the DNA fragment was purified by 1% agarose gel according to a general method ("Antibody", Harlow, E. and Lane, D. (1988), Cold Spring Harbor Lab. Press, New York). It was linked, using a T4DNA ligase, with a large DNA fragment of pBluescriptSK(+) (STRATAGENE) purified and digested by restriction enzymes BamH I and Xba I. The plasmid DNA was used to transform Escherichia coli (JM109), and the transformant was selected, to obtain a plasmid DNA (IgG^1 Bluescript). Then, expression vector pcDL-SR α296 was digested by restriction enzyme BamH I, and blunted at the termini by T4DNA polymerase treatment, and a Not I linker was linked. The large DNA fragment obtained by digesting it by restriction enzymes Not I and Xho I and the small DNA fragment obtained by digesting IgG^1 Bluescript by restriction enzymes Not I and Xho I were purified

according to a general method, and linked by T4DNA ligase. It was transformed into Escherichia coli (HB101) , and the transformant was selected, to obtain a plasmid DNA. Hereinafter this plasmid (IgG $_1$SRα) is called the human IgG^1 expression vector. In the following examples, since the basic protocol of gene manipulation is the same as above, the description will be simplified.

Please replace the paragraph bridging pages 38 and 39 with the following:

The template cDNA, primers, dNTPs and Taq polymerase were mixed in a PCR buffer, and the mixture was treated by a thermal cycler at 94°C for 1 minute for DNA denaturation, at 58°C for 2 minutes for annealing the primers and at 72°C for 3 minutes for elongating the primers. This treatment was performed for 30 cycles. The amplified DNA fragments of α4-2 and α4-3 were digested by Pst I and EcoR I respectively, or EcoR I and BamH I and sub-cloned into pBluescriptKS(+) (STRATAGENE), to prepare plasmid DNAs (hereinafter called α4-2 Bluescript and α4-3 Bluescript). Then, upstream of the α4-2 Bluescript, α4-1 was linked using Xba I and Stu I restriction sites, to prepare a plasmid DNA (hereinafter called α4-1-2 Bluescript).

Please replace the paragraph bridging pages 41 and 42 with the following:

Then, into the stabilized integrin β1·IgG heavy chain chimeric protein producing CHO cells, the integrin α4·IgG heavy chain chimeric protein expression vector was transfected according to the lipofectin method as described before. That is, integrin α4·IgGSRα and pSV2neo (BRL) were mixed at 10 : 1, and the mixture was mixed

with lipofectin reagent. The mixture was added dropwise into the cells. After 18 hours of dropwise addition, the mixture was cultured in the said first selective medium for about 2 hours, and the cells were dispersed by trypsin-EDTA treatment. The cells were suspended in a second selective medium (nucleic acid-free αMEM medium (GIBCO BRL) containing 10% FBS (GIBCO) and 1 mg/ml neomycin (GIBCO)), and on a 96-well plate (CORNING), resistant cells were selectively cultured for about 10 days. The amount of integrin α4·IgG heavy chain chimeric protein and the amount of integrin β1·IgG heavy chain chimeric protein produced in the culture supernatant solution were determined according to the ELISA method (described later), and a clone which produced both the chimeric proteins ~~by~~in almost the same amounts was picked up. The clone was cloned twice according to the limiting dilution method, ~~to be~~and stabilized as a clone capable of producing an α4·IgG heavy chain-β1·IgG heavy chain chimeric protein heterodimer complex.

Please replace the first full paragraph on page 48 with the following:

On the other hand, the immunoprecipitation pattern obtained by using the anti-integrin β1 antibody beads in the presence of 10 mM EDTA was the same as that in the presence of 1 mM $MgCl_2$, to clarify that the association between integrin α4•IgG heavy chain chimeric protein and integrin β1•IgG heavy chain chimeric protein does not depend on cations. The above results suggest that the eluted protein obtained in (3) of Example 6 was ~~certain~~certainly an α4•IgG heavy chain-β1•IgG heavy chain chimeric protein heterodimer

complex, and if the result of (4) of Example 6 is also taken into account, it is strongly suggested that the association between both the proteins is a stable association through a disulfide bond existing ~~between~~in the IgG heavy chains.

Please replace the heading on page 48 with the following:

(2) Examination ~~on~~of the structural stability of α4·IgG heavy chain-β1·IgG heavy chain chimeric protein heterodimer complex by sequential immunoprecipitation.

Marked-Up Version Showing Changes Made to the Claims

2. (Three Times Amended) A chimeric protein heterodimer complex, wherein a chimeric protein comprising (a) an α chain of an integrin and a heavy chain or light chain of an immunoglobulin and a chimeric protein comprising (b) a β chain of an integrin and a heavy chain or light chain of an immunoglobulin are ~~associated with~~bound to each other, and wherein the integrin α chain and β chain each have its function retained.

3. (Twice Amended) A chimeric protein heterodimer complex, according to claim 2, wherein ~~the~~said chimeric proteins comprise an α chain• immunoglobulin heavy chain-β chain • immunoglobulin heavy chain chimeric protein heterodimer complex, wherein a chimeric protein comprising the α chain of an integrin and the heavy chain of an immunoglobulin and a chimeric protein comprising the β chain of the integrin and the heavy chain of the immunoglobulin are ~~associated with~~bound to each other and stably associated with its function retained.

5. (Twice Amended) A chimeric protein heterodimer complex, according to claim 2 or claim 3, wherein the β chain of ~~an~~said integrin is β1, β2, β3, β4, β5, β6, β7 or β8.

25. (Twice Amended) A drug composition, comprising ~~a chimeric protein as stated in any one of claims 45-49 or~~ a chimeric protein heterodimer complex as stated in claim 2 or 3.

Please cancel Claims 24 and 45-49 without prejudice and without disclaimer of the subject matter contained therein.

Remarks

We have revised the specification as to form and have sharply amended the claims in an endeavor to promptly bring about the allowance of the case, and respectfully ask leave that these amendments be entered accordingly after final Action.

It is important in evaluating the instant invention to give full faith and credit to the Applicants' comments submitted under their Oath or Declaration, clearly stating that the matter of isolating and preparing an integrin with its function retained is very difficult. One reason for this is that since the association between an α chain and a β chain of an integrin is maintained non-covalently, the chains are easily disassociated during the process of isolation and preparation. This is pointed out in the Applicants' specification at the bottom of page 4, and throughout.

As is pointed out at the middle of page 7 of the Applicants' specification, no integrin with an α chain and a β chain structurally stably associated and with its function retained has ever been successfully prepared. The fact that the complex structure is unstable restricts the use of its molecule (page 7, lines 12-16).

We have introduced these important and sharp distinctions in the main claims and earnestly submit that these revisions present the claims in proper form for complete allowance.

Turning to the Gallatin publication, WO 95/17412, we note with appreciation the Examiner's agreement that Gallatin et al. do not teach that the integrin/Ig fusion proteins contain the particular α or β integrin chains recited in the claims.

The mere fact that certain integrin chains are known per se in the art does not in any sense amount to a disclosure or suggestion of the specific <u>combination</u> set forth in the Applicants' claims.

The Applicants have discovered a remarkable combination comprising a chimeric protein heterodimer complex. The chimeric protein comprises (a) an α chain of an integrin and a heavy chain or light chain of an immunoglobulin. It also comprises (b) a β chain of an integrin and a heavy chain or light chain of an immunoglobulin. These are bound to each other (as sharply distinguished from the previous definition calling for them broadly to be "associated with each other". Further, the claimed chimeric protein heterodimer complex is characterized by the fact that the integrin comprises stably associated α chain and β chain, and with its function retained after having been formed into the heterodimer complex. This is well supported in regard to "bound to" by the specification at page 28, line 21 to page 31, line 17 and elsewhere; and the retention of the function is specifically defined in the specification as filed, at page 7, lines 12-16 and elsewhere.

Comparable amendments have been made in Claim 3 and, of course, carry through to the dependent claims.

It would be in error, and contrary to the controlling decisions, to dismiss patentability solely because certain integrin chains "were known in the art". This falls far short of a teaching or disclosure by the art that the complete combination as defined in the now amended claims would produce new and beneficial results, contrary to the complete silence of the prior art that this might be the case. In essence, any rejection based on the simple fact that certain integrins were known in the art would be based upon an "invitation to experiment" which has long been struck down by the authorities as an incomplete basis for the determination of patentability or unpatentability.

In order to defeat patentability, even when applying the standards of §103, it is necessary to sustain the burden of proving that it would have been "obvious" to a person skilled in the art. There is no teaching in Gallatin that in any way suggests the specific combination set forth in the amended claims. Nor is there any suggestion that the

Applicants' specific combination, achieving a stably associated α chain and β chain, with its function retained, could even be contemplated in view of the scattered disclosures of the simple identifications of various integrins.

Reference has been made to an incomplete paragraph on page 7 of Gallatin; careful reading of the entire paragraph beginning at page 6, line 17 of the reference reveals that the disclosure, taken as a whole, simply refers to various purified and isolated polypeptides, fragments and variants thereof. In the entire paragraph there is no disclosure or suggestion of the complete combination defined by the amended claims.

Turning to the matter of Carter and Hori, taken together as a hypothetical "combination", all we find is that Carter teaches that immuno adhesions "have a variety of art-recognized uses", and the Examiner's comment that one of ordinary skill in the art would realize that these "have a variety of art-recognized uses". This, as in the case of Gallatin, simply amounts to an invitation by the Examiner for Carter et al. to perform some tests like those that were, in fact, performed by the Applicants and which resulted in the novel combinations defined in the amended solicited claims. There was no disclosed motivation to do this, because there is no indication of any reason for having attempted research with widely varying compounds, without a specific suggestion that a specific benefit would be obtained by doing so.

In regard to Hori, we appreciate the Examiner's courteous acknowledgment that Hori does not address the method or product of Carter et al. and does not disclose or deal with chimeric immuno adhesion molecules. Clearly, the teachings of Hori go no farther than to indicate that certain β1 integrin molecules "were known in the art as heterodimeric molecules", but this, of course, falls far short of any suggestion that any hypothetical combination might be made of Hori with Carter, or that any benefit would result therefrom.

For all the foregoing reasons, we respectfully submit that the amendments now made in the case present the case in allowable form. In view of the great novelty and advantage

of the creation of a chimeric protein heterodimeric complex, wherein the chimeric protein (a) comprises an α chain of an integrin and a heavy chain or light chain of an immunoglobulin and wherein the chimeric protein (b) comprises a β chain of an integrin and a heavy chain or a light chain of an immunoglobulin, wherein they are bound to each other, and wherein the integrin comprises stably associated α chain and β chain with its function retained. This is a highly meritorious invention fully justifying the grant of a patent, which is respectfully requested.

Further, we respectfully submit that none of the cited references teaches or suggests production of a chimeric protein heterodimeric complex of the invention. We invite the Examiner's attention to the fact that it was discovered for the first time in this invention that the chimeric protein heterodimer complex is applicable for pharmaceutical use as a substitute of adhesive platelets, as is shown in Example 22 using α2β1-IgG. Because of this highly surprising function of the claimed product, this invention is anything but obvious over the cited references.

In light of the foregoing, we respectfully submit that the entire application is now in condition for allowance, which is respectfully requested.

Respectfully submitted,

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